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STUDIES ON TICK-BORNE ENCEPHALITIS AND OTHER ARTHROPOD-BORNE VIRUS DISEASES

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Final Technical Report

By

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H. Hofmann, M. D., A. Radda, Ph. D.

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13. ABSTRACT

1. Distribution of tick-borne encephalitis virus (TBEV): six new foci have been discovered in Austria. TBEV is widespread in Switzerland. 2.TBEV control program: control of ticks and small mammals has met with some success. 3.Purification of arboviruses: accomplished with porous glass exclusion chromatography. 4.Synthesis of receptor substance TPI: Ten steps out of 22 have been completed. 5.Clinical studies: 288 cases of TBE have been diagnosed. 6.Virus infections: Langat virus causes chronic infection in mice which is not eliminated by Poly-I:C. 7.Serological studies: Antibodies against Tribec virus were detected occasionally in sera of cattle from Carinthia. Antibodies against Uukuniemi and other viruses were detected in birds. In Turkey, positive human and animal sera were found against TBEV, West Nile, and a group A virus. Among residents of West Cameroon positive human sera were found against group A and B viruses including O'nyongnyong, Chikungunya, Uganda S, Zika, Yellow Fever and Dengue II. 8.An unidentified virus was isolated from a migrating bird in Austria.

Key words: Arboviruses in Austria; tick-borne encephalitis eradication; tick-borne encephalitis ecology; virus receptor substance synthesis; arboviruses, concentration and purification; arbovirus infection, persistent; Langat virus; tick borne encephalitis in Switzerland; arboviruses in Turkey; arboviruses in Cameroon; arbovirus hosts, birds.

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Abstract

(I) Field studies of TBE led to the detection of 6 new TBE virus (TBEV) foci in Austria. Tick and mammal control program appear to be effective against virus cycle in nature. Survey with sera of Carnivora showed wide distribution of TBEV in Switzerland.

(II) Ten of 22 steps necessary for chemical synthesis of TBEV receptor TBI, have been completed. By means of exclusion chromatography with porous glass arboviruses can be purified and concentrated. TBEV has no immunosuppressive effect in mice. Langat virus, which belongs to TBE complex, causes chronic infection in mice and cannot be eliminated by interferon inducer Poly I:C.

(III) Clinical studies: 288 cases of TBE were diagnosed.

(IV) Surveys with sera of cattle and birds showed incidence of the tick-borne viruses "Tribec" and "Uukuniemi" in Austria.

(V) Unidentified virus was isolated from migrating bird after arrival in Austria.

(VI) Survey with human and animal sera indicated activity of at least one group A virus and of TBE and West Nile viruses in Turkey. Sera from residents of Cameroon had antibodies to several group A and B arboviruses including O'nyongnyong, Chikungunya, Uganda S, Zika, Yellow Fever and Dengue 2.

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CONTENTS

TICK-BORNE ENCEPHALITIS (TBE) AND OTHER GROUP B ARBO - VIRUSES

Field Studies

(1) Detection of new foci and attempts to eradicate the virus.

(1;1) Introduction.

Following the method of sending out questionnaires to patients with TBE we could find some new foci of TBE in Lower Austria in 1970 and 1971 (see last Annual Report (16)). Then also studies on the effectiveness of the organophosporus Gardona (R) against ticks were started. On the other hand we tried to reduce the small mammal populations in a focus in Carinthia in order to interrupt the natural cycle of TBE virus. Under the present contract (July 1971-July 1972) we continued the searching for new foci in Lower Austria, Burgenland, Carinthia and even in Styria and for the first time in the Tyrols. Field trials were done with another insecticide, namely with Malathion (R), which is also an organophosporus compound. The small mammal reduction program in Taggenbrunn, Carinthia, was continued.

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(1,2) Methods.

Ticks: Nymphs and adults of Ixodes ricinus were collected by flagdrapping and transported to the laboratory. The nymphs were homogenized in pools of 1-50 individuals, the adults in pools of 1-20 individuals, respectively. They were suspended in a medium consisting of PBS and 10% horse serum and inoculated intracerebrally into baby mice. The animals were observed for 18 days.

Malathion (R): Two field trials were done in order to develop a control program with this compound against ticks. In the spring of 1972 in a wood near the Danube at Mühlleiten, a few miles southeast of Vienna, and in ap afforestation near Hernstein we marked 8 fields of 25 m^2 each. Four of these fields were sprayed using the ULV (Ultra Low Volume) method at a concentration of 10 g Malathion (R) diluted in 5 liters of water per 25 m^2. Tick collections were made before spraying and on the 3rd and 8th day thereafter.

Gardona(R): Two field trials with Gardona (R) were carried out in July 1971 and in May 1972 in Hochosterwitz (Carinthia), which has been shown by us to be a permanent focus of TBE virus. The ground and the vegetation bordering the road to the fortress (i.e. the accessible part of the mountain on which the fortress is situated) were sprayed with 1 lb per acre of Gardona(R). In autumn the area was again searched for ticks. In the spring in 1972 we collected ticks again, and again sprayed the area in the same manner with Gardona(R)!

Small mammals: In the focus of Taggenbrunn, Carinthia, eradication of the virus by trapping of small mammals was attempted. So far, 5 excursions were done in 1971 and one in 1972. About 120 small mammal traps were set up for 1 to 3 nights.

(1,3) Results.

New foci in different parts of Austria: According to the informations obtained from patients with TBE, ticks were collected in different areas in Lower Austria, Burgenland, Carinthia and Styria. For the first time investigations on the possible occurrence of TBE virus in the Tyrols were conducted although no cases of TBE have been reported in this province. The results can be seen in Tables 1-4. Altogether 20 virus strains could be isolated from ticks collected at 98 different places. New foci were found in Lower Austria (Enzesfeld), Jauling/Enzesfeld, Hirtenberg), Burgenland (Allhau), Carinthia (Micheldorf) and Styria (Graz) (see Fig.1). So far we failed to isolate virus from ticks collected in the Tyrols.

The 3 foci found in the area of Hirtenberg-Enzesfeld are of special interest because in these foci many persons contracted infection. Therefore, in future studies we will try to interrupt the virus cycle in nature by spraying of these foci with insecticides.

Malathion(R): The results of the field study with Malathion(R) are presented in Table 5 and in Fig.2. A statistical evaluation done with the χ^2-test revealed that the number of ticks in the fields did not differ significantly prior to the treatment with Malathion(R). By contrast, after spraying with the compound there was a very significant difference between the number of ticks collected in the treated and in the nontreated fields. In Mühlleiten the tick population was reduced by approximately 95% seven days after the treatment. In Hernstein where the undergrowth is rather dense the even distribution of Malathion was not very easy. Nevertheless we observed a reduction of the tick population of about 85%.

Gardona(R): The first field trial with Gardona(R) in an established focus of TBE virus was carried out on July 8,1971, in Hochosterwitz (Carinthia). In July before the spraying we were able to isolate 4 strains of virus from 272 ticks collected there (Table 1). In autumn the area was again searched for ticks as it is shown in Table 1. From a total of 524 specimens of Ixodes ricinus not a single virus strain could be isolated. After collecting 430 nymphs and 43 adults of Ixodes ricinus in May 1972 we sprayed again the area bordering the road to the fortress with Gardona(R). Virus isolation experiments with these ticks were not successful, indicating that the virus cycle may indeed have been interrupted by our previous lowering of the density of ticks with Gardona(R).

Small mammals: In Taggenbrunn (Carinthia) we continued the small mammal control program with the aim of eradicating the virus in this focus. In 1971, in April, when the first excursion was made after the winter not a single mouse could be trapped. It is apparent from Table 6 that by summer the small mammal populations had somewhat built up and probably as a result of our control efforts - as a rule the highest number of free-living mice are found in October and November - had decreased again by autumn. In May of 1972 we again found a very low population density of mice. It is of special interest that in this year, 1972, no virus could be isolated from ticks although 264 nymphs and 19 adults were collected and tested for the presence of virus (see also Table 7).

Although it is too early to draw a definite conclusion this could possibly mean that we are on the way of reaching our goal of interrupting the virus cycle in the focus.

(1,4) Discussion and Conclusions.

The high number of new foci of TBE virus enables us to conduct now further studies on methods to eradicate the virus in nature. Now we will try to interrupt the virus cycle on the one hand by spraying the foci from the ground or from the air with Gardona (R) or with Malathion (R) and on the other hand by reducing the population density of the small mammal species in certain foci. In connection with the results of this program our first tests are very encouraging and we hope, that at the end of this year the effectiveness of our methods will further be substantiated.

(1,5) Summary.

In search of endemic areas of TBE in different provinces of Austria 6 new foci in Lower Austria, Burgenland, Carinthia and Styria were found by virus isolations from ticks. The effectiveness of Malathion(R) against ticks (Ixodes ricinus) was proved in two field trials. A reduction of between 85 % and 95 % of the tick populations was achieved.

In order to eradicate the virus in a well known permanent focus in Hochstorwitz Gardona (R) was sprayed in autumn 1971 and in spring 1972. After spraying no virus could be isolated from ticks collected in this area.

The control program of small mammals in Taggenbrunn was continued. The population densities of these animals were kept low and subsequently also in this permanent focus we failed to isolate virus from ticks, collected there in 1972.

(2) Survey in Lower Austria with sera of forest workers.

In the hemagglutination-inhibition (HI) test a survey was conducted with sera of forest workers from different districts in Lower Austria. The purpose of this study was to improve our knowledge on the distribution of TBE virus in this province but also to find a group of persons who are at high risk of infection. It is intended to perform a field trial in volunteers as soon as the first batch of an inactivated vaccine will be available, hopefully, some time during 1972. This batch is being made for us by a laboratory in Great Britain where we have sent 3 strains of TBE virus (tick suspensions). This laboratory has had some experience in the production of a vaccine against Louping ill. This disease is caused by a virus that is very closely related to TBE virus.

The results of the survey are presented in Table 8. It can be seen that of the 830 sera tested 101 (12.2%) had antibodies to the virus. A field trial is considered in those districts where the percentage of workers with antibodies exceeds 10%.

(2,1) Summary.

A survey was conducted with sera of forest workers from different districts in Lower Austria. Thus, our knowledge on the distribution of TBE virus in this province was improved and groups of persons, who are at high risk of infection, were found. It is intended to perform a field trial as soon as an inactivated vaccine will be available.

(3) Survey with sera of Carnivora from Switzerland.

In July 1970, the Institute of Veterinary Bacteriology and Parasitology, University of Bern, received the brain of a dog from Hallau (Kanton Schaffhausen) for diagnosis of rabies. An agent could be isolated from the dog which proved to be TBE virus. This was the first isolation of this virus in Switzerland, where in 1965 we had diagnosed a human case of TBE and, therefore, already then had claimed that the virus must be endemic also in this country (14). Since then a number of human cases were reported by other workers.

We were contacted by Drs.Steck and Wandeler from the Institute mentioned above and asked to conduct a survey with sera of Carnivora that had been shot during the rabies eradication program in Switzerland.

A totals of 519 sera of foxes, 20 of badgers, 20 of beech-martens and one of a polecat was received and tested in the HI-test against TBE virus. Of 560 sera, 91 were positive.

The latter were then also examined for antibodies against this virus in the tissue culture neutralization test. Of these, 15 were toxic for the cells and 76 gave a positive result.

It is a well known fact that sera from animals that are shot may become contaminated with nonspecific inhibitors for arboviruses such als bile. Since all animals had been killed by shooting we were not convinced about the validity of our results. In order to pick up nonspecific inhibitors all 91 positive sera were tested in the HI-test against Semliki and Sindbis antigens of group A and Yellow Fever, Dengue 2 and West Nile antigens of group B.

48 sera only reacted with TBE antigens, 21 were also positive with other group B viruses and 22 sera gave a positive test with group B and group A viruses. Under these circumstances we feel that only the HI-test with those 48 sera reacting exclusively with TBE virus, should be considered as a valid result due to specific antibodies.

In Fig.3 the geographic distribution of the samples is shown. From it it is striking that positive as well as negative sera are distributed more or less over the whole area under investigation. However, considering the home range of foxes which, according to the experiences of our Swiss colleagues, can be 50 km or more, many of these animals were shot a long distance away from the focus where they had acquired the infection.

A less random distribution is seen when the results are listed according to the age of the Carnivora (Table 9). Of the 31 foxes aged between 1 1/2 and 4 months none was found to have antibodies against TBE virus. Then a number of positive sera increasing with age were observed. As foxes are very good indicators for the presence of TBE virus in larger regions it can be stated with certainty that TBE virus must be more widely distributed in Switzerland than hitherto known.

(3,1) Summary.

Of 560 sera of Carnivora shot in Switzerland 48 specimens were found to contain antibodies against TBE virus in both the HI and the neutralization test. The geographic distribution of the positive sera shows that TBE virus must be more widely distributed in Switzerland than hitherto known.

Experimental Laboratory Investigations

(1) Studies on the receptor substance for TBE virus and other arboviruses of group B.

(1,1) Introduction and earlier results.

From brain-lipids, the receptor substance for group B arboviruses could be isolated (8,9,22). This substance proved to be a triphosphoinositide (TPI). It was a very strong inhibitor of the hemagglutination (HA) as well as of the infectivity of group B arboviruses (9,15). It reacted with TBE virus in a two step reaction. An initial reversible step, caused mainly by electrostatic attraction, is followed by a second one, caused by stronger forces which are not any more susceptible to the interaction of electrically charged molecules (16).

(1,2) Preparation of receptor substance.

TPI is now prepared in our laboratory as a routine procedure. We use a method of extraction, precipitation and chromatography which has been extensively described in the report of 1970 (15). Some refinements were introduced (16) and now we are able to obtain from 1000 g monkey brain 300 to 400 mg TPI in form of its Ca-salt.

(1,3) Chemical composition of TPI-Ca preparation.

The composition of our preparations was now estimated more exactly by quantitative photometry of thin layer chromatograms. These chromatograms were obtained by applying the method of GONZALEZ-SASTRE and FOLCH-PI (10), using chloroform-methanol-4N ammonia 9:7:2 as solvent and "Kieselgel HR" (Merck) with 0.1% oxalate as adsorbent (15). The intensity of the spots, stained with DITTMER's reagent (6), was now measured quantitatively with a JOYCE "Chromoscan". The areas beneath the extinction graphs were measured with a planimeter (geometrical integration). By dividing the integrated graphs by the number of phosphorus atoms in the formula of the substance forming the measured spot, values corresponding to the molar amount of each substance were obtained; due to the big difference of the relative concentrations of the main product (TPI) and the impurity (phosphatidylserine=PS), it was necessary to measure two series of dilutions. From one (Table 10) the ratio of TPI to DPI could be calculated to be 100 : 9.88; the other (Table 11) yielded the ratio of DPI to PS as 100 : 7.32. From these figures the percentage of TPI, DPI and PS in our product was calculated to be 90.40%, 8.95% and 0.65%, respectively.

(1,4) Chemical synthesis of intermediate products on the way for a total synthesis of TPI.

In cooperation with the Institute for Pharmaceutical Chemistry, there are also experiments under way to obtain a synthetical receptor substance. The main difficulty is the synthesis of the sterically right triphosphoinositol. Direct phosphorylation of inositol proved unsuccessful and the synthesis had to be started from aceto-bromoglucose. In a series of 21 steps, the final product, TPI, should be reached. Presently, the hydrolysis of step 9:2.3 di-o-allyl-6-(deoxy) 6-nitro-β-benzylglucoside to 2.3 di-o-allyl-6-(deoxy) 6-nitroglucose is investigated. The last mentioned compound is expected to be transferred into 1-deoxy-1-nitro-4.5-di-o-allyl-inositol by treatment with Ba-hydroxide. From this first inositol derivative 2,3,6-tribenzyl-4.5-di-o-allyl-myoinositol shall be obtained in five further steps. This compound should be able to combine with diacyl-glycerol and $POCl_2$ and, after phosphorylation and removal of the shielding groups, should yield finally TPI.

(2) Concentration and purification of arboviruses.

(2,1) Concentration of TBE virus by an aqueous two phase system.

It was tried to concentrate and to purify TBE virus using a two phase system, containing polyethylene glycol (PEG) and dextran (1). A similar system has been used already for the concentration of Japanese encephalitis virus (19).

Brains of infected baby mice were extracted with a borate saline buffer of pH 9 and treated with protamin sulfate (4). To such a virus suspension PEG (MW 6,000) and dextran (MW 110,000) was added to a final concentration of 10% and 0.5% respectively. From 30 ml total solution about 0.5 ml dextran rich phase separated which contained practically all of the added virus. This resulted in an approximately 50-fold concentration of the original virus preparation (Table 12). Using tissue culture fluid, the virus apparently disappeared. It seemed to be adsorbed onto a precipitate from which it could not be eluted again.

(2,2) Purification of arboviruses by chromatography on porous glass.

It was considered to treat arboviruses with enzymes to increase the HA-titre of badly hemagglutinating preparations, especially of viruses from the Bunyamwera supergroup. To obtain a good action of the enzymes, the virus batches have to be purified prior to the enzyme treatment in order to remove material which could possibly compete as substrate for the enzyme with the adsorbed impurities on the surface of the virus particles. It was also adviseable to develop a purification procedure in order to separate the virus from the enzyme after the treatment.

A very promising technique seemed to be chromatography on a recently developed medium: controlled porous glass (12). The principle is the same as in gel-chromatography. However, the pores of the glass can be made of nearly equal diameter and all interconnecting. This causes that a seperation which would need 20 to 30 hours using an organic gel like Sephadex can be performed in as many minutes, using CPG. Furthermore, columns filled with glass can be sterilized either chemically or by autoclaving before and after use. The technique has been used already to purify plant viruses (12) and bacteriophages (11).

Preliminary experiments were performed with a column filled with glass granules with a pore diameter of 332 Å. The column had 11 mm diameter and was 100 cm long. For these tests, West Nile-Virus was used as a model. The virus was extracted from infected baby mouse brains with 10 vol. of borate saline of pH 9, centrifuged at 20,000 g for 30 minutes, then ultracentrifuged at 105,000 g for 3 hours. The sediment was homogenized in about 1/10 of the original volume of the same buffer and the undissolved residue was sedimented at 3,800 g for 30 minutes and discarded. The supernatant had an HA-titre of about 20,000. 2 ml of this partly purified preparation were applied to the column and eluted with borate saline, pH 9, at a flow rate of 2 ml/min. Monitoring with UV at 280 Å revealed a very sharp peak at 30 ml which contained all the HA-activity. A broad peak with a shoulder at 66 ml and a maximum at 80 ml represented smaller particles and molecules. About equal amounts of viral and impurity protein were contained in this preparation as could be judged from the integration of the curves. An excellent separation of virus from protein molecules was also obtained when an equal volume of 4% albumin was admixed to the virus before it was applied to the column. Again a very sharp peak with HA-activity occurred at 38 ml. The albumin appeared as a very broad peak with a maximum at 62 ml. The original impurities could be observed as a shoulder at 80 ml (Fig.4). In this case, the ratio of viral to nonviral protein was 1:7. Therefore, this method seemed to be very appropriate for a separation of arboviruses from added enzymes. In these experiments however, the yield of the viral HA was only 10 to 20 per cent. Because of the obvious advantages of this method, we continued our experiments. After trying different elution media and a glass with a smaller pore diameter (240 Å), it was finally possible to obtain yield as large as 75 to 85 per cent of HA. Now we use a 0.05 M Tris buffer, pH 8.2 + 0.1 M NaCl (Tris saline) and a coating of the porous glass with polyethylene glycol (MW=20,000) (13).

Our model virus (TBE) was extracted from infected baby mouse brains with borate saline, pH 8.5, and then treated with protamin sulfate (4). This resulted in precipitation of acidic impurities. By CPG-chromatography further impurities and also the excess of the added protamin could be separated from the virus (Fig.5). This procedure could be repeated in a way that a new batch of virus was applied when the protein-impurities of the previous sample were just leaving the column. With 4 ml portions of virus applied to the column and a flow rate of 2.5 ml/min it was possible to

purify 20 ml of TBE virus in little more than three hours (Fig.6).

Further experiments with coated CPG were performed: Supernatants of low-speed centrifugations of homogenates from mouse brains infected with Sindbis virus and with West Nile virus were applied to the column without previous ultracentrifugation or precipitation. Remarkably, the 20 to 60 fold amount of viral HA was obtained by CPG chromatography alone (Table 13). However, with the very poorly hemagglutinating Tahyna virus (Bunyamwera supergroup) no increase of the HA-titre could be observed. It is hoped that the intended enzymatic treatment of such preparations prior to chromatography will yield reasonably good hemagglutinins.

(2,3) Summary.

The receptor substance for arboviruses of group B can be prepared from monkey brain in good yield. Thin layer chromatograms of such preparations were evaluated quantitatively by photometry. The product consisted of 90.4% TPI, 8.9% DPI, and 0.7% PS. But also chemical synthesis of TPI was undertaken. Starting from glucose, step 10 of a planned chain of 22 steps could already be prepared.

TBE virus could be purified and concentrated 40-100 fold by partition in an aqueous two phase system containing PEG and dextran. In order to separate arboviruses from enzymes to be added for purification, exclusion chromatography on porous glass was investigated. Model experiments showed that added albumin and protamin could be removed from arboviruses by this technique. Coating the glass with PEG resulted in a dramatic increase in the yield of viral HA. When low speed supernatants of mouse brains, infected with group A or B arboviruses were applied to porous glass columns, the purified virus showed 20 to 60 times more HA than the supernatant. It was not possible to obtain hemagglutinating preparations of Bunyamwera supergroup viruses by chromatography alone and enzymatic treatment had to be considered.

(3) Chronic Infection of Mice with Langat Virus.

DENK and KOVAC (5) studied the histopathology of Langat virusinfection in mice. They observed seven months p.i. residual encephalitis in animals, which had survived

infection without any clinical signs of disease. This chronic encephalitis caused by a member of the TBE complex of group B arboviruses was investigated by us in detail by virological and serological methods.

(3,1) Persistence of Langat virus in the brain of mice after s.c.infection.

Eighty mice (strain GP, NIH), weighing 15 g, were infected subcutaneously with 1000 LD_{50} (for baby mice i.c.) of Langat virus (strain TP 21). 47 mice survived infection and were considered as being chronically infected. Beginning 3 weeks p.i., at weekly intervals, 4 mice were killed and their sera and brains were pooled.

The brain suspensions (1:10 dilutions in PBS) were tested for the presence of virus, CF-antigen and interferon. The sera were searched for antibodies, virus and interferon.

As can be read in Table 14, traces of virus were found in some of the brain suspensions until the end of the experiment (13th week p.i.). Brains also contained CF-antigen of Langat virus in titers ranging from 1:8 to 1:32. Interferon was neither detected in brain nor in serum. Also no virus was found in the serum, obviously due to its content of antibodies, which were demonstrated in titers from 1:80 to 1:320. These antibodies were of the IgG-type as revealed by the 2-mercapto-ethanol-test (18). This indicates that the infection was no longer in the acute stage.

(3,2) Behaviour of Langat virus after i.c.infection and of TBE virus after s.c.infection.

Ten mice each were infected i.c. with 1000, 100, 10 and 1 LD_{50} (for baby mice i.c.) of Langat virus. All animals but 4, which had been infected with 1 LD_{50}, died. These survivors neither showed virus nor complement fixing Langat antigen in the brains. Thus application of the virus by the intracerebral instead of the subcutaneous route does not cause chronic infection. Besides, much smaller quantities of virus are needed for lethal encephalitis as compared with those necessary by the peripheral route.

In the next experiment, 20 mice were s.c.infected with 10 LD_{50} (for baby mice i.c.). of TBE virus (strain

Hypr). Eight mice survived; however, neither the virus nor TBE antigen could be detected in the brains. Thus, TBE virus, an agent closely related to Langat virus, does not cause chronic infection in mice.

(3,3) Susceptibility of chronically infected animals for a challenge infection with Semliki Forest virus.

A group of 46 mice infected chronically with Langat virus as well as a control group of 29 noninfected mice with the same weight and age were challenged with 3 LD_{50} of Semliki Forest (SF) virus four weeks after Langat virus infection. In Table 15 it can be seen that 78% of the chronically infected and 62% of the control mice succumbed SF infection. Average survival time was 4.9 and 4.8 days respectively. From that we conclude, that chronic Langat virus infection neither prevents infection with a second virus nor does it make the mice more susceptible to it.

(3,4) Influence of the interferon inducer Poly I:C on the chronic Langat virus infection.

Interferon was neither found in sera nor in the brains of chronically infected mice, therefore we tried to eliminate the virus from the brain by the interferon inducer Poly I:C, which is very effective against TBE in mice (17) and which is known to induce interferon not only in the serum but also in the brain (3). The experiment is summarized in Table 16. Out of 31 chronically infected mice 10 were sacrificed and their brains tested individually for virus and antigen: 8 mice showed antigen and 5 therefrom also the virus. Eleven of the remaining 21 mice were treated each i.p. with 200 µg Poly I:C, 10 mice remained nontreated and served as controls. Four days after Poly I:C application antigen was demonstrable in the brains of all 5 mice tested, virus was found 4 times. Of the 5 corresponding control mice 4 showed the antigen and 2 also the virus. After 7 days antigen was found in the brains of five treated as well as of five nontreated mice. Virus could be detected in 2 treated and in 1 nontreated mouse. Thus by means of Poly I:C neither virus nor the antigen could be eliminated from the brains of chronically Langat virus infected mice.

(3,5) Discussion.

Our experiments clearly indicate, that Langat virus may persist after peripheral infection in the brain for a long time. Similar results have been presented by PRICE (20), who infected mice with Kyasanur Forest Disease virus, which is also a virus of the TBE complex. However, virus persistence seems not to be a general feature of these viruses, because after peripheral infection with TBE, virus did not persist in the brains of mice. But also after i.c. Langat virus infection of mice virus persistence could not be observed.

We assume that after peripheral infection due to extraneural virus replication nonspecific defense of the organism is well stimulated. Thus the brain is protected and virus cannot multiply to the extent, that is necessary for the development of encephalitis. After i.c.infection nonspecific defense is not stimulated or too late and the animal therefore always dies after infection.

An important mechanism of nonspecific defense against virus infection is the interferon response of the host, which theoretically could be responsible for the above mentioned resistance of s.c.infected mice against Langat virus. However, this does not seem to be the case because interferon never was found in chronically infected mice, which also were equally susceptible for a challenge infection as noninfected animals. Besides, Poly I:C had no influence on the content of virus and antigen in the brain.

Recently, attention has been focused on the persistence of measles virus in human brain and its relation to Subacute Sklerosing Panencephalitis. The results of our study provide some evidence that, at least in the brains of mice, also arboviruses may reach a stat. of chronic infection. In view of these findings one wonders whether this could not happen in the brain of man too. This the more, because we were frequently told by the clinicians that occasionally TBE can take a rather protracted course with long lasting disorders in the EEG.

(3,6) Summary.

The chronic Langat virus infection of mice was studied virologically in detail. The virus was recovered from the brains until the end of the study (13th week). Also

CF antigen was found in the brains in titers ranging from 1:8 to 1:32. In sera no virus but HI antibodies of the IgG-type were found against this virus. Interferon was neither detected in the brains nor in the serum. The chronically infected mice never did show any sign of disease and were equally susceptible for a second infection with SFV as normal mice. Their brains cannot be freed from the virus by application of the interferon inducer Poly I:C.

(4) Study on the possible immunosuppressive effect of the TBE virus.

It has been published that some viruses - predominantly oncogenice viruses - have an immunosuppressive effect.

In the present study the influence of TBE virus was investigated by us on the antibody-producing systems in mice. This was done by injecting a suspension of goose erythrocytes intraperitoneally into infected and noninfected animals and comparing the titer of goose cell agglutinating antibodies that had been formed in both groups of mice by the fourth day thereafter.

Initially the concentration of goose erythrocytes in 0.2 ml saline was determined in noninfected mice giving rise to sufficiently high titers. The resulty may be seen in Table 17. Due to these results a concentration of 2% was chosen for all the experiments.

Experiment 1: Three groups of 10 mice each were infected with about 10 LD_{50} of TBE virus, strain "Hypr". One, 2 and 3 days thereafter mice of one group were immunized, and 4 days after immunization the animals were bled. Noninfected controls were immunized and bled in the same manner. Antibody titers against goose erythrocytes in all groups of animals (infected and noninfected) were 1:256.

Experiment 2: In this experiment mice were infected with a very high dose of virus ($10^5 LD_{50}$). Four groups were immunized, 1,2,3 and 4 days p.i. respectively. Although those mice which had received red blood cells 4 days p.i. were bled in a moribund state they exhibited the same levels of antibodies against the goose cells as the con-

trols. Thus, TBE virus does not appear to have an immunosuppressive effect, although it multiplies in antibody producing tissues of infected mice.

(4,1) Summary.

By immunizing mice with goose-erythrocytes at different days after infection, TBE virus was not found to have an immunosuppressive effect.

Studies on patients

(1) Diagnostic studies.

Since the beginning of 1971 we usually diagnose TBE by means of the 2-mercaptoethanol-test, which was described in last year's Final Technical Report ('5). This test gives excellent results and is well accepted by the physicians at the hospitals, because it makes an early diagnosis possible. Only in a few cases a second serum sample is required and the CF-test must be performed.

The 288 cases of TBE diagnose by those methods in the year 1971 in the different provinces of Austria are listed in detail in Table 18. For comparison, Table 19 gives the results of the first three months of the season 1972. So far, there seems to be about the same incidence of TBE in 1972 as in 1971. However, it will be of great importance, if the summer will be hot and dry or cool and rainy.

(1,1) Summary.

In the year 1971, 288 cases of TBE had been recorded. From the first cases recorded in 1972 it is likely that during this year 1972 the disease will have about the same incidence as last year.

TRIBEC VIRUS

(1) Survey for antibodies against Tribec virus with sera of cattle.

Tribec virus belongs to the Kemerovo group of arboviruses and was first isolated from ticks in Czechoslovakia. Some Czech workers claim that this agent may be the cause of encephalitis in man.

Because of the possible clinical importance of this virus and its transmission by the same tick, Ixodes ricinus, which is the vector of TBE virus, we attempted to get some information on its incidence in Austria by means of a serologic survey with sera of cattle. These domestic animals are known to develop antibodies against this virus.

So far, a total of 1825 sera was tested in the neutralization test using 100-300 $TCID_{50}$ of the virus and chick embryo cells. Antibodies were detected in 14 sera indicating the rare incidence of Tribec virus in Austria.

From Table 20 it is striking that all positive sera but 2 came from cattle from Carinthia. Only one positive serum each was in the samples from Salzburg and Burgenland. Titers of positive sera ranged between 1:10 and 1:40.

(1,1) Summary.

Antibodies against Tribec virus were found in sera of cattle from Carinthia. Sera of cattle from other districts tested (Styria, The Tyrols, Upper Austria, Salzburg, Burgenland) were negative with only two exceptions.

SURVEYS ON THE INCIDENCE OF ARBOVIRUSES

(1) Investigations on the role of birds as hosts of arboviruses in Austria.

(1.1) Introduction.

It is well known that birds may represent important hosts of arboviruses. Due to the fact that many species migrate over large distances every year, they may also introduce viruses which do not normally circulate in certain regions, particularly in moderate zones.

So far, in Austria no investigations on the role of birds as hosts of arboviruses have been carried out. In order to elucidate their importance from the arbovirological aspect, we started a field program in the Neusiedlersee area during fall 1970 which was continued in 1971 and 1972.

First in fall 1970, we directed our attention to starlings (Sturnus vulgaris), a species which occure in very high population densities in the Neusiedlersee area. Many attempts were made to capture a representative number of this species using several methods at day and at night. It proved, however, to be extremely difficult to obtain larger numbers of blood samples of starlings; despite of a high expense only 32 sera could be collected. We therefore tried to get permission for capturing all bird species occurring in the reed zone of the Neusiedlersee area which we obtained in 1971. Thus, numerous bird species could be netted in reasonably high numbers during various seasons in 1971 and 1972.

In total, so far the following investigations were carried out:

(a) Virological and serological survey with 32 sera of starlings.

(b) Virological and serological investigations with 488 sera of 21 bird species (migrating and non-migrating) collected during autumn 1971.

(c) Serological survey with 125 sera of 6 bird species collected during late winter 1972.

(d) Virological and serological investigations with 149 sera of 16 migrating species during spring 1972.

(1,2) Methods.

Birds were captured with Japanese mist nets.

In the case of the starlings the nets were placed in open areas as well as in the reed zone in the vicinity of the sleeping sites of these birds in the Western part of the Neusiedlersee area. As soon as one starling had been netted the others shunned this locality, so that we were forced continuously to change the capture places. We also tried to force the swarms into the nets - at day by driving a car over the meadows behind the swarms, at night by startling them by boat or by foot. This latter method was also unsatisfactory as the sleeping sites are in most cases situated in places which are inaccessive to a boat (too much reed), although the water level sometimes reaches a height of 1 m. At any rate, the success was very poor.

As we got the permission also to capture birds other than starlings in 1971, all further collections of birds were carried out in the reed zone near the village Neusiedl in the Northeastern part of the lake. The nets were put up early after sunrise and removed in the evening.

With the exception of the starlings which were bled from the wing vein, blood was taken from all other birds from the jugular vein. Birds were marked and released immediately after puncture. Some birds were recaptured some days later; these specimens were however released without taking a second blood sample.

A small part of the blood was immediately frozen in dry ice and then kept at -80°C until virus isolation experiments were done. The main part of the blood (about 0.05 to 0.2 ml) was immediately diluted in 0.5 ml of PBS, kept in ice for some hours and then frozen at -20°C until serological examination.

Virus isolation trials were carried out by i.c.inoculation of the blood into baby mice. The mice were observed over a period of two weeks.

Some of the starling sera were tested for neutralizing antibodies against Tahyna virus in a tissue culture

of the cell line GMK-AH-1 with methods previously described (2).

All other sera were tested for hemagglutination inhibiting antibodies according to the reference method described by CLARKE and CASALS (4). The sera were treated with acetone and tested in a dilution of 1:10 against 4 to 8 units of certain antigens (see below). They were regarded as positive when they reacted in this dilution; due to the fact that already the blood samples had been diluted immediately after puncture, the real dilutions were higher, ranging from 1:20 to 1:50, or even more.

(1,3) Virological and serological survey with sera of starlings.

During the period from September 23 until October 15, 1970, blood samples of 32 starlings (Sturnus vulgaris) were collected. From these 23 samples (Nr.1-23) were tested for neutralizing antibodies against Tahyna virus, 29 sera (Nr.4-32) were tested for hemagglutination inhibiting antibodies against the following antigens: Yellow Fever (YF), Dengue II, West Nile (WN), TBE, Sindbis and Semliki.

In three samples antibodies against arboviruses could be detected. Serum Nr.5 reacted positively in the hemagglutination inhibition (HI)-test against YF and Dengue II; serum Nr.9 reacted positively in the HI-test against Dengue II and in the neutralization test (NT) against Tahyna virus; serum Nr.12 has neutralizing antibodies against Tahyna virus.

From none of the blood samples virus could be isolated.

(1,4) Virological and serological survey with sera of 21 migrating and non-migrating bird species captured in autumn.

On September 3,1971, we started collections of blood samples of birds netted in the reed zone of the Neusiedlersee and continued these captures until October 22,1971.

During this period a total of 488 blood samples from 21 bird species was obtained.

No virus could be isolated from any of the samples tested.

A serological survey was done with all sera by testing them in the HI-test against the following antigens: TBE, WN, Tukuniemi, Chikungunya, Semliki, Sindbis, Calovo and Tahyna. The results are shown in Table 21.

(1,5) Serological survey with 6 bird species during winter.

In order to investigate the question whether birds which normally do not leave Central Europe may act as hosts of arboviruses, that are endemic in the Neusiedlersee area, blood samples were collected of birds netted in the reed zone of the Neusiedlersee area during the period from February 16 to April 8,1972.

Altogether 125 individuals belonging to 6 species were captured. The sera were tested in the HI-test against the antigens mentioned under (2,4). The results of these studies are shown in Table 22.

(1,6) Virological and serological survey with 16 migrating birds just at arrival in Austria in spring.

In order to study the question of a possible introduction of arboviruses by birds from tropical and subtropical regions a field study was carried out in the reed zone of the Neusiedlersee during the period from March 15 to May 17,1972. In this investigation only those birds were included which had newly arrived from abroad, so that the detection of a possible viremia could be expected. Altogether, 149 birds belonging to 16 species were captured. The blood samples were tested for virus and for hemmagglutination inhibiting antibodies against the antigens mentioned under (2,4).

The results of this investigation are shown in Table 23.

(1,7) Discussion.

In the investigations described above, altogether 794 birds belonging to 28 species (of 12 families) were included from which so far 639 individuals were tested for virus and 786 for antibodies.

Out of the blood of a Robin (Erithacus rubecula) a strain of a virus was isolated. The agent could serially be propagated in baby mice. Reisolation from the blood specimen was successful. A crude antigen was prepared from the brains of mice infected with the 3rd passage of this virus and tested in the complementfixation test with sera against the following viruses: TBE, WN, Uukuniemi, Tribec, Calovo and Tahyna. None of the sera reacted with the agent. Thus the virus does not appear to be identical with any of the arboviruses which are known to be endemic in Austria.

Table 24 summarizes the results obtained in the HI-tests. All positive sera will be tested for neutralizing antibodies in near future. From Table 24 it will be seen that most positive reactions occurred with Uukuniemi virus. ERNEK et al. (7) also found in 5 of 25 sera of a number of bird species captured in Slovakia hemagglutination inhibiting antibodies against Uukuniemi. Our results indicate that this virus probably also occurs in Austria.

Also antibodies found against TBE and Calovo viruses might be traced back to infections acquired in Central Europe while most other positive findings (Chikungunya, Semliki, Sindbis, WN, YF, Dengue II) found in migrating birds (Locustella luscinioides, Acrocephalus melanopogon, Acrocephalus scirpaceus, Acrocephalus arundinaceus, Sturnus vulgaris) may reflect prior infections with group A and B viruses in Africa or in the Mediterranean region.

Of particular interest is, however, the finding of hemagglutination inhibiting antibodies against Semliki virus in a Bearded Tit (Panurus biarmicus) and in Blue Tits (Parus caeruleus) thus indicating that these birds had been infected with a group A arbovirus. The populations found in Austria normally do not leave Central Europe during winter, very rarely do they migrate, however, to the Northern parts of the Mediterranean region. At any rate, no arbovirus of group A has so far been isolated in Europe, and the results obtained give, therefore, a further hint to the (perhaps occasional) occurrence of a group A arbovirus on the continent.

Furthermore, the finding of antibodies against WN virus in a Blue Tit cannot be clearly interpreted. Perhaps this particular bird had already once been in the Me-

diterranean region, where WN virus is endemic; on the other hand it cannot be excluded that WN virus may occasionally occur in the Neusiedlersee area, where its vector Culex modestus is found.

Finally it should be stressed that in none of the 754 sera tested in the HI-test antobodies against Tahyna virus could be detected while among 23 starlings examined in the Neutralization test antibodies were found in two specimens. By contrast, in experimental studies SIMKOVA (24) found that young starlings (1-6 days old) are not susceptible for Tahyna virus and neither develop viremia nor neutralizing antibodies. Thus, the question arises whether elder individuals are susceptible to the virus or whether the two positive tests may be due to nonspecific virus inhibitors in the sera. Furthermore, the question, whether birds are involved in the circulation of the Tahyna virus remains open.

(1,8) Summary.

During the period from September 1970 until May 1972 blood was taken from 794 birds belonging to 28 species which were captured in the Neusiedlersee area in the east of Austria.

So far, 639 individuals were tested for virus and 786 for antibodies; from these, 754 samples were tested for hemagglutination inhibiting antibodies against Uukuniemi, Calovo, Tahyna, Chikungunya, Semliki, Sindbis, TBE and WN only. Of the remaining 32 sera (all from starlings) 29 samples were tested for hemagglutination inhibiting antibodies against YF, Dengue II, WN, TBE, Sindbis and Semliki, and 23 samples for neutralizing antibodies against Tahyna virus.

Out of the blood of a robin a strain of a virus (probably an arbovirus) was isolated which could, however, not yet be identified.

Hemagglutination inhibiting antibodies were found in the following birds (in brackets: antigens against which antibodies were detected/number of positive samples): Spotted Crake (TBE/1), Green Sandpiper (Calovo/1), Savi's Warbler (Sindbis/1, TBE/1, WN/2), Sedge Warbler (TBE/1), Reed Warbler (Uukuniemi/4, Chikungunya/2, Semliki/5, Sindbis/1, WN/3), Great Reed Warbler (Chinkungunya/1), Bearded Tit

(Semliki/1), Robin (Uukuniemi/1), Blue Tit (Uukuniemi/5, Semliki/4, WN/1), Reed Bunting (Uukuniemi/2, Chikungunya/1) and Starling (YF/1, Dengue II/2), Moustached Warbler (Chinkungunya/1, Sindbis/1, TBE/1, WN/2). In addition, neutralizing antibodies against Tahyna virus were found in two starlings.

The result of the study appears to indicate the presence of Uukuniemi virus in Austria, although most of the other positive findings can be explained by prior infections acquired in Central Europe or (in the case of migrating birds) in the Mediterranean region or in Africa.

Of particular interest are, however, reactions with antigens of group A arboviruses with the blood of birds which do not migrate or, at least, not farther than the Mediterranean region. This is a hint for the occurrence of a group A arbovirus in Europe.

(2) Studies on the activity and ecology of arboviruses in Turkey.

In 1965, Dr.Radda took part in an expedition to Turkey in order to collect zoological material for the Museum of Natural History in Vienna. Then he was also able to collect more than 200 blood samples of domestic animals. The results of a serological survey with these sera showed that in the surroundings of Ankara West Nile (WN) virus or an agent very closely related is active. In the south-eastern part of Anatolia, in the Vilayet Hatay, he found animals with antibodies against one virus of group A and one of group B. From the results of the neutralization test the latter seemed to be Tick-borne encephalitis (TBE) virus (21).

Very similar results were obtained by SERTER (23) who also made investigations on the activity of arboviruses in Turkey. He diagnosed three human cases of TBE with serologic methods and could demonstrate the presence of antibodies in human beings against TBE, West Nile, Dengue II (D II), Tahyna and Sindbis viruses in the surroundings of Izmir.

Because of these findings we felt that further studies on the incidence of arboviruses in Turkey were indicated. In 1971, Dr.Radda was awarded a medical fellowship by the Council of Europe in order to conduct studies on the

activity and ecology of arboviruses in Anatolia. He worked from September 20 until November 20,1971, and again from March 17 until April 15,1972, at the Department of Microbiology and Infectious Diseases of the Medical Faculty, Ege University in Bornova/Izmir.

At first, collections of sera from human beings (healthy people as well as outdoor patients) were made. A total of 270 serum specimens were obtained and shipped to our laboratory for testing (see Table 25). In addition, 263 sera from sheep deriving from 8 different places (Isparta, Konya, Tire, Canakkale, Aliaga, Milas, Menemen, Edremit) which had been slaughtered in Izmir were also tested (see Table 26). The results summarized in Table 25 provide further evidence for the occurrence of at least one virus of group A and two viruses of group B in the Izmir area. The group A agent appears to be related to Semliki virus while one of the group B agents must be very close to or identical with WN virus. Surprisingly none of the human sera reacted with TBE virus in the hemagglutination inhibition (HI)-test. However, among the sera from sheep 5 were positive when tested against this virus.

In order to catch small mammals in the surrounding of Kemalpasa, a small town about 30 km east of Izmir, where one human case of TBE had been observed (22), small mammal traps were set up in different habitats. In 14 trapping nights (491 trap-units) 82 individuals of different species of small mammals were trapped (see Table 27). These animals obviously were not caught in a focus of TBE virus as it is shown by the lack of antibodies against this antigen (Table 25). Yet 4 sera gave positive results (3 Mus musculus, 1 Apodemus spec.) in the HI-test with the Semliki or two group B antigens. Dr.Radda also collected 90 human sera from residents of Istanbul and 95 sera from Ankara in different hospitals. The sera of both groups of humans yielded similar results and provided some evidence for the activity of one or, perhaps, two viruses of group B (see Table 25).

Sera that had been positive in the HI-test against TBE or other group B viruses were also tested against TBE virus in the neutralization test using L cells for the antibody assay. This was done in order to prove with this more specific test that at least some of the antibodies against group B viruses actually were due to infections

with this virus. Thus in 9 sera neutralizing antibodies against TBE virus were found (see Table 28).

During fall of 1971 it was hardly possible to collect any ticks because of their inactivity at that time of the year. However, in spring 1972, a total of 782 ticks, mostly larvae, nymphs and adults of Ixodes ricinus, but also some specimens of Haemaphysalis punctata, Rhipicephalus bursa and Hyalomma aegyptium could be collected in pine forests and shrubs along fields on places where also small mammals had been trapped during the fall of 1971. Virus isolation experiments from these ticks were not successful (see Table 29). Finally the plant associations were analysed in the places where field studies on ticks and their small mammal hosts were done (see Table 30).

(2,1) Summary.

Sera from humans, sheep and small mammals were investigated for antibodies against A and B arboviruses. About 5% were positive. It seems that one of the three probable causative agents is related to Semliki Forest virus, the other two appear to be West Nile and Tick-borne encephalitis virus. Attempts at isolation of TBE virus from ticks collected in Turkey were not successful.

(3) Survey with human sera from West Cameroon.

During a zoological collecting trip from January until February, 1971, Dr.Radda was able to do some medical studies at the Presbyterian General Hospital in Nanyemen, West Cameroon. These studies were granted by a fellowship of the "Notring Wissenschaftlicher Verbände Österreichs". In order to perform a serological survey on the activity of arboviruses in West Cameroon 173 serum specimens from outdoor patients of the local population were collected and shipped to our laboratory in Austria.

Sera were treated with acetone to remove nonspecific inhibitors in the hemagglutination inhibition (HI)-test. The test was done following the classical procedure described by CLARKE and CASALS (4). The following antigens, prepared from sucrose-acetone treated baby-mouse brains, were used: Semliki Forest (SF), Sindbis (Sind.), Yellow Fever (YF), Murray Valley Encephalitis (MVE), West Nile (WN), Dengue II (D 2), Tick-borne Encephalitis (TBE).

The results of the HI-test can be seen in Table 31. Sixty-seven sera from a total of 173 sera tested gave positive reactions. Many sera showed cross reactions with more than one antigen.

All the 40 sera of the first group which were positive in the HI-test were tested again in the neutralization test (NT) in green monkey cells (strain GMK-AHI),2 against the following viruses: U'nyongnyong, Chikungunye, Zika, Uganda S. These viruses are known to occur in Africa. The results can be seen in Table 32. Titers of hemagglutination inhibiting and neutralizing antibodies of all positive sera are listed in Table 33. Upon reading the results of the HI-test it will be noted that a few sera reacted to a low titer with Semliki and Sindbis antigens which are group A arboviruses. These are probably cross reactions which were possibly caused by infections, with agents closely related to or identical with U'nyongnyong and Chikungunya viruses, as it can be seen from the results with the more specific NT. The pattern of hemagglutination inhibiting antibodies against the group B viruses (YF, MVE, WN, D 2, TBE) tested is somewhat puzzling. However, from our experience and that of other workers it is a reasonable assumption that a number of group B viruses are active in the area where the sera were collected. This would also explain the high titers against MVE virus, although this agent does not occur in Africa. On the other hand, some of antibodies against YF virus (see sera Nr.22,42 and 57) could be caused by infections with this virus. None of the persons, whose blood was included in the survey, had previously been vaccinated against Yellow Fever. Besides, at least one type of Dengue virus appears to be endemic in the area under investigation (see sera 21,33,80 and 92). In addition, Uganda S and Zika viruses are probably present in West Cameroon, as it can be concluded from the result of the NT.

(3,1) Summary.

A serological survey on 173 sera of the residents of West Cameroon revealed the activitiy of at least two arboviruses of group A, probably U'nyongnyong and Chikungunya as well as four viruses of group B including Uganda S, Zika, Yellow Fever and a member of the Dengue viruses.

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Table 1: Number of ticks (Ixodes ricinus) collected in different areas in Burgenland (B), Lower Austria (L.A.) and Carinthia (C) and virus isolation therefrom 1971.

Location, Date	Number of nymphs collected (pools)	strains isolated	Number of adults collected (pools)	strains isolated
Deßburg/Baumgarten (B.) June 16	144 (7)	-	16 (3)	-
			1*	
Hied near Raabs (L.A.) June 10-11	378 (19)	-	88 (19)	-
Zero/Fahrafeld (L.A.) June 24	251 (13)	-	14 (3)	-
Neudörfl/Püttaching (L.A.) July 6	335 (17)	-	50 (10)	-
	36* (2)			
Enzenfeld (L.A.) Sept.19	266 (13)	-	26 (5)	1
	2*			
Hirtenberg (L.A.) Sept.19	108 (5)	-	17 (3)	2
St.Veit/Triesting (L.A.) Sept.19	146 (7)	-	7 (1)	-
Michaldorf (C.) Oct.2	48 (2)	1	4 (1)	-
Wieting A (C.) Oct.1	32 (2)	-		
Wieting B (C.) Oct.3	44 (2)	-	13 (3)	-

Table 1: (Flow sheet, cont.)

Location, Date	Number of nymphs collected	(pools)	strains isolated	Number of adults collected	(pools)	strains isolated
Hochosterwitz (C.)						
July 7	272	(14)	4	20	(4)	-
Sept. 11-12	206	(10)		15	(3)	-
Oct. 1	195	(10)		8	(2)	-
Taggenbrunn (C.)						
July 8	205	(10)	1	39	(8)	-
Sept. 11-12	194	(10)	-	10	(2)	-
Oct. 1	228	(11)	1	6	(1)	1

33-1

* = Haemaphysalis concinna

Table 2: Number of ticks (Ixodes ricinus) collected in different areas in Burgenland (B), Lower Austria (L.A.) and Carinthia (C.) and virus isolation therefrom 1972.

Location, Date	number of nymphs collected	(pools)	strains isolated	number of adults collected	(pools)	strains isolated
Apetlon (B.) April 6-7	3	(1)	-	10**	(2)	-
				18		-
Allhau II (B.) April 7	184	(9)	3	43	(6)	1
Allhau I (B.) April 8	229	(11)	-	16	(2)	-
Siegreben (B.) April 6 and 8	58	(3)	-	35	(3)	-
				6**	(2)	-
Hohenegg I (L.A.) April 30	117	(5)	-	9	(1)	-
Hohenegg II (L.A.) April 30	264	(13)	1	27	(4)	-
Jauling/Enzesfeld (L.A.) May 10	412	(20)	1	50	(5)	-
Hernstein II (L.A.) May 12	372	(19)	-	10	(1)	-
Taggenbrunn (C.) May 13	264	(14)	-	19	(2)	-

Table 2: (Flow sheet, cont.)

Location, Date	Number of nymphs collected	(pools)	strains isolated	Number of adults collected	(pools)	strains isolated
Hochosterwitz (C.)						
May 13	430	(22)	-	43	(5)	-
Hirtenberg (L.A.)						
May 24-25	840	(42)	2	97	(10)	1
Enzesfeld (L.A.)						
May 24-25	101	(5)	-	46*	(5)	-
				1**	(1)	-

* = Haemaphysalis concinna

** = Dermacentor pictus

Table 3: Number of ticks (Ixodes ricinus) collected in different areas in Styria (St) and in Tyrol (T) and virus isolations therefrom in 1972

Location, Date	Number of nymphs collected	(pools)	strains isolated	adults collected	(pools)	strains isolated
Graz, Autobahn (St.) June 3	196	(4)	1	31	(4)	-
Hechenberg (T.) June 6	119	(6)	-	4	(1)	-
Matzen (T.) June 6	2	(1)	-	14	(2)	-
Telfs-Zirl (T.) June 6	25	(1)	-	1	(1)	-
Schwarz-See (T.) June 6	1	(1)	-	7	(1)	-
Unterperfuß (T.) June 6	8	(1)	-	42	(3)	-
Ötz I (T.) June 6	12	(1)	-	11	(1)	-
Ötz II (T.) June 6	2	(1)	-	6	(1)	-
Zirl-Telfs (T.) June 6	90	(5)	-	8	(1)	-
Windau (T.) June 6	2	(1)	-	5	(1)	-

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Table 4: Number of ticks (Ixodes ricinus) collected 1971 and 1972 in different areas and virus strains isolated therefrom

Virus strain No.	Pool size	Location	Date
34253	5 females	Enzesfeld	Sept. 19, 1971
34260	5 males	Hirtenberg	Sept. 19, 1971
34262	5 females	Hirtenberg	Sept. 19, 1971
34352	28 nymphs	Micheldorf	Oct. 3, 1971
33540	20 nymphs	Hochosterwitz	July 7, 1971
33547	20 nymphs	Hochosterwitz	July 7, 1971
33576	20 nymphs	Hochosterwitz	July 7, 1971
33578	12 nymphs	Hochosterwitz	July 7, 1971
33586	20 nymphs	Taggenbrunn	July 8, 1971
34324	20 nymphs	Taggenbrunn	Oct. 1, 1971
34326	4 females + 2 males	Taggenbrunn	Oct. 1, 1971
36443	20 nymphs	Allhau II	April 4, 1972
36445	20 nymphs	Allhau II	April 4, 1972
36449	20 nymphs	Allhau II	April 4, 1972
36455	7 females + 1 male	Allhau II	April 4, 1972
36505	28 nymphs	Hohenegg II	April 30, 1972
36593	20 nymphs	Jauling/Enzesfeld	May 10, 1972
36765	20 nymphs	Hirtenberg	May 24, 25, 1972
36793	20 nymphs	Hirtenberg	May 24, 25, 1972
36801	5 females + 5 males	Hirtenberg	May 24, 25, 1972
36854	54 nymphs + 5 adults	Graz/Autobahn	June 3, 1972

Table 5: Results of tick collections before and after Malathion treatment in Mühlleiten and Hernstein II.

	Field No.	May 5 Number of nymphs	(adults)	May 8 Number of nymphs	(adults)	May 12 Number of nymphs	(adults)
Mühlleiten:							
Control fields:	1	15	(3)	1		11	(2)
	3	4		6		2	(4)
	5	22	(3)	1		10	(2)
	7	16	(1)	11	(3)	14	(5)
		57	(7)	19	(3)	37	(13)
Treated fields:	2	13	(2)	-		1	(1)
	4	12	(2)	1		2	
	6	18	(5)	1		-	
	8	19	(7)	-		-	(1)
		62	(16)	2		3	(2)
Hernstein II:							
Control fields:	1	26	(1)	30		26	(3)
	3	23	(1)	25	(1)	37	(1)
	5	12	(1)	11	(1)	16	(4)
	7	21	(1)	17		29	
		82	(4)	83	(2)	108	(8)
Treated fields:	2	141	(2)	20		13	
	4	80	(1)	5	(1)	7	
	6	48	(2)	7		5	
	8	21	(1)	9	(2)	4	
		290	(6)	41	(3)	29	

Table 6: Results of small mammal trapping in Taggenbrunn (July 1971 - June 1972).

Excursion No.	Date	Trapped animals
1	July 7-8 1971	16 Apodemus spec. 5 Clethrionomys glareolus 1 Microtus spec.
2	August 9-11 1971	14 Apodemus spec. 2 Clethrionomys glareolus 3 Microtus spec.
3	Sept. 10-12 1971	8 Apodemus spec. 1 Microtus spec.
4	Oct. 1-3 1971	8 Apodemus spec. 1 Clethrionomys glareolus
5	Nov. 5-8 1971	11 Apodemus spec. 5 Clethrionomys glareolus 3 Microtus spec. 2 Sorex araneus
6	May 13-15 1972	4 Apodemus spec. 1 Microtus spec.

Table 7: Number of ticks (Ixodes ricinus) collected in Taggenbrunn in three subsequent years and number of virus strains isolated therefrom.

Excursion date	Number of nymphs collected	Number of strains isolated	Number of adults collected	Number of strains isolated
Sept. 1-5, 1969	204	-	64	6
May 27, 1970	668	17	93	8
July 8, 1971	205	1	39	-
Sept. 11-12, 1971	194	-	10	-
Oct. 1-3, 1971	288	1	6	1
May 13-15, 1972	264	-	19	-

Table 8: Results of HI-tests with sera of forest workers and TBE virus.

District of Lower Austria	Number of workers tested	Number of workers with antibodies
Baden	164	36 (22%)
Wr.Neustadt	64	11 (17%)
Neunkirchen	174	11 (6%)
Preßbaum	46	1
St.Pölten	154	25 (16%)
Lilienfeld	165	9 (5%)
Scheibbs	63	8 (13%)
T o t a l	830	101

Table 9: Antibodies against TBE virus in sera of foxes according to age.

Age	Number of sera tested	No HI antibodies	Positive only in TBE HI test	Positive in TBE- and other group B-HI test	Positive in TBE and in group B and group A-HI test
1 1/2-4 months	31	30	0	1	0
4-8 months	132	113	10	6	3
8-12 months	83	67	9	3	4
older than 12 months	266	221	27	11	9
T o t a l	514	431	46	21	16

Table 10: Quantitative evaluation of thin layer chromatograms of polyphosphoinositides I. : Ratio TPI : DPI.

A_{TPI}	A_{DPI}	A_{PS}	$A_{TPI}/3$	$A_{DPI}/2$	TPI : DPI
316	14.0	0.8 *	105.3	7.0	100 : 6.65
161	10.0	n.d. *	53.7	5.0	100 : 9.32
359	22.8	1.2 *	119.6	11.4	100 : 9.54
184	17.3	n.d. *	61.3	8.6	100 : 14.01

Mean ratio TPI : DPI = 100 : 9.88

A_{TPI} : Area between base line and extinction graph of TPI spot

A_{DPI} : Area between base line and extinction graph of DPI spot

A_{PS} : Area between base line and extinction graph of PS spot

* : Value too small to be measured exactly

n.d. : Not determined quantitatively.

All values arbitrary units.

Table 11: Quantitative evaluation of thin-layer chromatograms of polyphosphoinositides II. : Ratio DPI : PS.

A_{TPI}	A_{DPI}	A_{PS}	$A_{DPI}/2$	DPI : PS
662*	153	6.5	76.5	100 : 8.50
685*	264	6.5	132.0	100 : 4.93
582*	176	7.5	88.0	100 : 8.52
675*	220	8.5	110.0	100 : 7.27

Mean ratio DPI : PS = 100 : 7.02

A_{TPI} : Area between base line and extinction graph of TPI spot

A_{DPI} : Area between base line and extinction graph of DPI spot

A_{PS} : Area between base line and extinction graph of PS spot

* : Value too large to be measured exactly

All values given in arbitrary units

Table 12: Concentration of TBE virus by an aquous two phase system.

			Experiment No.			
			1	2	3	4*
Original	Volume (ml)	:	25.5	25.5	25.5	25.5
	HA-titre	:	2,560	5,120	2,560	12,800
	Units**	:	65,000	130,000	65,000	326,000
PEG-phase	Volume (ml)	:	29.5	29.5	29.5	29.5
	HA-titre	:	32	32	16	128
	Units**	:	940	940	470	3,770
	Yield	:	1.5%	0.7%	0.7%	1.2%
Dextran-phase	Volume (ml)	:	0.4	0.5	0.5	0.5
	HA-titre	:	256,000	256,000	204,800	512,000
	Units**	:	102,000	128,000	102,000	256,000
	Yield	:	>100%	98%	>100%	79%
Concentration-factor		:	100x	50x	40x	20x

* The original was already purified once by the two phase system

** Arbitrary units resulting from multiplication of titre by volume

Table 13: Increase of HA-titre of some arbovirus preparations by CPG-chromatography.

	Volume	HA-titre	HA-units
Sindbis virus:			
Applied to column	2 ml	512	1024
Eluate, Fraction No.:			
16	2 ml	64	128
17	2 ml	4,096	8,192
18	2 ml	8,192	16,384
19	2 ml	1,024	2,048
20	2 ml	256	512
21	2 ml	128	256
22	2 ml	64	128
			27,649
West Nile virus			
Applied to column	2 ml	8	16
Eluate, Fraction No.:			
15	2 ml	--	--
16	2 ml	64	128
17	2 ml	256	512
18	2 ml	128	256
19	2 ml	32	64
20	2 ml	16	32
21	2 ml	8	16
22	2 ml	4	8
23	2 ml	2	4
			1,020

Table 14: Persistence of Langat virus in the brains of mice.

Week	Brain			Serum		
	Virus	Interferon	CF antigen	Virus	Interferon	HI antibodies
3	+	--	1: 8	-	-	1:160
4	+	-	1:32	-	-	1:320
5	+	-	1:16	-	-	1: 80
6	+	-	1:16	-	-	1:160
7	+	-	1: 8	-	-	1:320
8	+	-	1:16	-	-	1:320
9	+	-	1: 8	-	-	1:320
10	-	-	1:32	-	-	1:160
11	-	-	1:16	-	-	1:320
12	-	-	1:16	-	-	1:320
13	+	-	1:32	-	-	1:320

Table 15: Susceptibility of chronically infected animals for a challenge infection with Semliki Forest virus.

Group	Number of mice infected with SFV	Number of mice succumbed to SFV infection (%)	Average survival time
Mice chronically infected with Langat virus	46	36 (78%)	4.9 days
Control	29	18 (62%)	4.8 days

Table 16: Langat virus in the brains of mice with chronic infection after treatment with Poly I:C.

Group	Number of mice	Treatment	Time of testing	Number of mice with virus in the brain	Number of mice with CF antigen in the brain
1	10	no	prior to Poly I:C	5	8
2	5	Poly I:C	4 days after	4	5
3	5	no	as group No.2	2	4
4	6	Poly I:C	7 days after Poly I:C	2	5
5	5	no	as group No.4	1	5

Table 17: Immunization of mice with goose erythrocytes (0.2 ml/mouse).

Concentration of goose erythrocytes in %	Titer of antibodies against goose erythrocytes 4 days after injection
0.005	neg.
0.01	neg.
0.05	neg.
0.1	1:2
0.5	1:4
1.0	1:8
5.0	1.128
10.0	1:256

Table 18: Cases of TBE in 1971 in Austria.

Province	April	May	June	July	August	September	October	November	December	TOTAL
Vienna	1/0/0*	2/1/0	8/0/0	13/0/0	4/0/0	4/1/1	2/1/0	1/0/0		35/ 3/1
Lower Austria	1/0/0	8/0/0	21/3/0	35/0/0	11/1/2	3/1/0	1/1/0	1/0/0		81/ 6/2
Carinthia		2/1/0	19/2/0	33/6/0	15/0/0	14/2/0	4/1/0	1/0/0	0/1/0	88/13/0
Styria			7/2/0	8/0/0	4/2/0	2/1/0				21/ 5/0
Upper Austria		3/0/0	4/1/0	5/1/0	7/1/0	2/0/0	5/0/0			26/ 3/0
Burgenland			1/0/0	1/0/0		1/1/0				3/ 1/0
Salzburg		1/0/0	1/0/0		1/0/0					3/ 0/0
TOTAL	2/0/0	16/2/0	61/8/0	95/7/0	42/4/2	26/6/1	12/3/0	3/0/0	0/1/0	257/31/3

* TBE : confirmed (2 ME)/confirmed (CI)/uncertain (HI positive, ME- and CF-Tests inconclusive)

Table 19: Cases of TBE in 1972 until June in Austria.

Province	April	May	June
Vienna		3/0/0	14/0/0
Lower Austria	1/0/0*	10/2/1	16/4/1
Carinthia		3/2/0	17/1/1
Styria			
Upper Austria	1/1/0	4/0/0	15/0/1
Burgenland	1/0/0	1/0/0	1/0/0
Salzburg			
The Tyrol			1/0/0
T o t a l	3/1/0	21/4/1	64/5/3

* TBE confirmed by 2-ME/CF/uncertain (HI positive, 2-ME and CF-tests uncertain)

Table 20: Antibodies against Tribec virus in sera of cattle from different provinces of Austria.

Province	Number of sera tested	Number of positive sera
Styria	680	0
The Tyrol	225	0
Upper Austria	180	0
Salzburg	254	1
Carinthia	473	12
Burgenland	13	1
T o t a l	1825	14

Table 21: Virological and serological survey with sera of migrating and non-migrating bird species.

Species	Number of blood samples tested for virus/virus isolation	Number of sera tested for antibodies/number of +) positive sera
Fam. ARDEIDAE		
1. Little Bittern (Ixobrychus minutus)	1/-	1/-
Fam. RALLIDAE		
2. Water Rail (Rallus aquaticus)	1/-	1/-
3. Spotted Crake (Porzana porzana)	1/-	1/1 TBE
Fam. SCOLOPACIDAE		
4. Snipe (Gallinago gallinago)	2/-	2/-
Fam. ALCEDINIDAE		
5. Kingfisher (Alcedo atthis)	2/-	2/-
Fam. HIRUNDINIDAE		
6. Swallow (Hirundo rustica)	1/-	1/-
Fam. MOTACILLIDAE		
7. White Wagtail (Motacilla alba)	2/-	2/-
8. Blue-headed Wagtail (Motacilla flava)	1/-	1/-
Fam. MUSCICAPIDAE		
9. Savi's Warbler (Locustella luscinioides)	6/-	6/-
10. Moustached Warbler (Acrocephalus melanopogon)	40/-	40/ 1U, 1CH, 1WN
11. Sedge Warbler (Acrocephalus schoenobaenus)	17/-	17/-

Table 21: (Flow sheet, cont.)

12. Marsh Warbler (Acrocephalus palustris)	3/-	3/-
13. Reed Warbler (Acrocephalus arundinaceus)	187/-	187/ 2U, 2CH, 5SFD, 1WN
14. Great Reed Warbler (Acrocephalus arundinaceus)	15/-	15/1CH
15. Bearded Tit (Panurus biarmicus)	37/-	37/1SFD
16. Chiffchaff (Phylloscopus collybita)	1/-	1/-
17. Robin (Erithacus rubacula)	1/-	1/-
Fam. REMIZIDAE		
18. Penduline Tit (Remiz pendulimus)	36/-	36/-
Fam. PARIDAE		
19. Blue Tit (Parus caeruleus)	52/-	52/2SFD, 1WN
20. Great Tit (Parus major)	1/-	1/-
Fam. EMBERIZIDAE		
21. Reed Bunting (Emberiza schoenicius)	81/-	81/1U, 1CH
T o t a l	488/-	488 4U, 5CH, 8SFD, 1TBE, 3WN

+) For abbreviations see Table 21 A.

Table 21 A: Abbreviations.

TBE	=	Tick borne-encephalitis
U	=	Uukuniemi
CH	=	Chikungunya
SFD	=	Semliki Forest Disease
WN	=	West Nile
C	=	Calovo
Sind	=	Sindbis
YF	=	Yellow Fever
MVE	=	Murray Valley Encephalitis
D_2	=	Dengue II
O'nyong=		O'nyongnyong
UgS	=	Uganda S

Table 22: Serological survey with birds captured during winter.

Species	Number of sera tested for antibodies/number of positive sera +)
Fam. TROGLODYTIDAE	
1. Wren (Troglodytes troglodytes)	1/-
Fam. MUSCICAPIDAE	
2. Bearded Tit (Panurus biarmicus)	3/-
Fam. REMIZIDAE	
3. Penduline Tit (Remiz pendulimus)	23/-
Fam. PARIDAE	
4. Blue Tit (Parus caeruleus)	69/5U, 2SFD
5. Great Tit (Parus major)	1/-
Fam. EMBERIZIDAE	
6. Reed Bunting ++) (Emberiza schoeniclus)	28/1U

+) Abbreviations see Table 21 A

++) Reed Buntings found in Austria during winter have usually spent the summer in other parts of Europe situated north of Austria.

Table 23: Virological and serological survey with birds returning from tropical and subtropical hibernation areas.

Species	Number of birds captured so far/ Number of blood samples tested for virus/virus isolation	Number of sera tested for antibodies/Number of positive sera +)
Fam. SCOLOPACIDAE		
1. Snipe (Gallinago gallinago)	1/-	1/-
2. Redshank (Tringa totanus)	3/3/-	3/-
3. Green Sandpiper (Tringa ochropus)	1/1/-	1/TBE, C
Fam. MOTACILLIDAE		
4. Blue-headed Wagtail (Motacilla flava)	1/1/-	1/-
5. White Wagtail (Motacilla alba)	3/3/-	2/-
Fam. MUSCICAPIDAE		
6. Savi's Warbler (Locustella luscinioides)	10/8/-	9/1TBE, 1Sind, 1WN
7. Moustached Warbler (Acrocephalus melanopogon)	9/8/-	9/1TBE, 1Sind, 1WN
8. Sedge Warbler (Acrocephalus schoenobaenus)	8/8/-	8/1TBE
9. Reed Warbler (Acrocephalus scirpaceus)	40/24/-	37/2U, 1Sind, 2WN
10. Great Reed Warbler (Acrocephalus arundinaceus)	35/24/-	32/-

Table 23: (Flow sheet, cont.)

Species	Number of birds captured so far/ Number of blood samples tested for virus/virus isolation	Number of sera tested for antibodies/Number of positive sera +)
11. Garden Warbler (Sylvia borin)	1/1/-	1/-
12. Blackcap (Sylvia attricapilla)	1/1/-	1/-
13. Chiffchaff (Phylloscopus collybita)	1/1/-	1/-
14. Willow Warbler (Phylloscopus trochilus)	2/2/-	2/-
15. Robin (Erithacus rubecula)	18/18/virus not yet identified	18/1U
Fam. EMBERIZIDAE		
16. Reed Bunting (Emberiza schoeniclus)	16/15/-	15/-
T o t a l	150/118 1 virus not yet identified	141/3U, 1C, 3Sind, 4TBE, 4WN

+) Abbreviations see Table 21 A

Table 24: Hemagglutination inhibiting antibodies against arboviruc in birds captured in Austria.

Species	Number of sera tested	Number of sera reacting in the HI-test against: U	C	T	CH	Semi.	Sind.	TBE*	WN	YF	D' II
Fam. ARDEIDAE											
1. Little Bittern (Ixobrychus minutus)	1									not done	
Fam. RALLIDAE											
2. Water Rail (Rallus aquaticus)	1									"	
3. Spotted Crake (Porzana porzana)	1							1		"	
Fam. SCOLOPAZIDAE											
4. Snipe (Gallinago gallinago)	3									"	
5. Redshank (Tringa tonatus)	3									"	
6. Green Sandpiper (Tringa ochropus)	1		1					1		"	
Fam. ALCEDINIDAE											
7. Kingfisher (Alcedo atthis)	2									"	
Fam. HIRUNINIDAE											
8. Swallow (Hirundo ruotica)	1									"	

Table 24: (Flow sheet, cont.1)

Species	Number of sera tested	Number of sera reacting in the HI-test against: U	C	T	CH	Seml.	Sind.	TBE+	WN	YF	D..II
Fam. MOTACILLIDAE											
9. Blue-headed Wagtail (Motacilla flava)	2										not done
10. White Wagtail (Motacilla alba)	4										"
Fam. TROGLODYTIDAE											
11. Wren (Troglodytes troglodytes)	1										"
Fam. MUSCICAPIDAE											
12. Savi's Warbler (Locustella luscinicides)	15						1	1	1		"
13. Moustached Warbler (Acrocephalus melanopogon)	49				1		1	1	2		"
14. Sedge Warbler (Acrocephalus schoenobaenus)	25							1			"
15. Marsh Warbler (Acrocephalus palustris)	3										"
16. Reed Warbler (Acrocephalus scirpaceus)	224	4			2	5	1		3		"

Table 24: (Flow sheet, cont.2)

Species	Number of sera tested	Number of sera reacting in the HI-test against: U	C	T	CH	Seml.	Sind.	TBE+	WN	YF	D II
17. Great Reed Warbler (Acrocephalus arundinaceus)	47				1					not done	
18. Garden Warbler (Sylvia borin)	1									"	
19. Blackcap (Sylvia attricapilla)	1									"	
20. Bearded Tit (Panurus biarmicus)	40					1				"	
21. Chiffchaff (Phylloscopus collybita)	2									"	
22. Willow Warbler (Phylloscopus trochilus)	2									"	
23. Robin (Erithacus rubecula)	19	1								"	
Fam. REMIZIDAE											
24. Penduline Tit (Remiz Pendulimus)	59									"	

Table 24: (Flow sheet, cont.3)

Species	Number of sera tested	Number of sera reacting in the HI-test against: U	C	T	CH	Seml.	Sind.	TBE*	WN	YF	D II
Fam. PARIDAE											
25. Blue Tit (Parus caeruleus)	121	5				4			1	not done	
26. Great Tit (Parus major)	2									"	
Fam. EMBERIZIDAE											
27. Reed Bunting (Emberiza schoeniclus)	124	2			1					"	
Fam. STURNIDAE											
28. Starling (Sturnus vulgaris)	29									1	2
T o t a l	783	12	1	-	5	10		3	5	7	1	2

+ Abbreviations see Table 21 A

Table 25: Results of the hemagglutination inhibition tests with sera from Turkey.

Antigen	Number of positive sera from Izmir (human)	Izmir (sheep)	Kemalpasa (small mamm.)	Istanbul (human)	Ankara (human)
Semliki Forest Disease	6	-	1	-	-
Sindbis	-	-	-	-	-
West Nile	17	4	-	1	4
Tick-borne encephalitis	-	5	-	-	-
Dengue II	1	2	2	-	-
Murray Valley encephalitis	7	4	2	7	3
Yellow Fever	4	2	-	1	3
T o t a l	23*/270	12*/263	4*/82	8*/90	5*/95

Number of positive/investigated sera

* Some of the sera showed cross reactions with more than one antigen

Table 26: Results of the survey with 263 sheep sera from Western Anatolia.

Geographic region	Number of sera investigated	Number of positive sera	Titers of the positive sera in the HI +
Kenya	17	1	WN 1:20
Isparta	92	4	2 WN 1:20, 3 TBE 1:20, 2 YF 1:20
Tire	30	1	WN 1:10
Canakkale	21	-	
Edremit	23	-	
Aliaga	24	1	MVE 1:10
Menemen	33	3	2 MVE 1:10, D2 1:10
Milas	23	2	2 TBE 1:10, 1 MVE 1:10, 1 D2 1:10
T o t a l	263	12	4 WN, 5 TBE, 2 YF, 4 MVE, 2 D2

+ Abbreviations see Table 21 A

Table 27: Results of small mammal trappings in Kemalpasa and results of HI-tests.

Species	Number of sera tested	Number of positive sera +
Mus musculus spicilegus	37	3 (SFD, MVE, D2)
Apodemus spec.	28	1 (MVE)
Cricetulus migratorius	3	-
Crocidura spec.	12	-
Crocidura suaveolens	1	-
Suncus etruscus	1	-
T o t a l	82	4

+ Abbreviations see Table 21 A

Table 28: Results of the neutralization test with TBE virus.

Serum Nr.	Species	Deriving	Antibody titer
254	Homo	Izmir	1:80
73/71	Ovis	Isparta	1:80
98/72	Ovis	Milas	1:40
281	Homo	Istanbul	1:20
284	Homo	Istanbul	1: 5
286	Homo	Istanbul	1:10
292	Homo	Istanbul	1: 5
62	Homo	Ankara	1: 5
70	Homo	Ankara	1:80

Table 29: Results of tick collections near Kemalpasa and Belkave

Species and developmental stage	Habitat Macchia (Kemalpasa)	Habitat Pinetum (Forest, Belkave)	Total
Ixodes ricinus			
Larvae	70	271	341
Nymphs	51	304	355
Adults	6	18	24
Hyalomma aegyptium			
Adults	-	5	5
Rhipicephalus bursa			
Adults	-	2	2
Haemaphysalis punctata			
Larvae	52	-	52
Adults	-	3	3
T o t a l	179	603	782

Table 30: Plant communities in habitats of ticks and their small mammal hosts in possible foci of TBE virus in Kemalpasa and Belkave.

Species	Frequency of species in the habitat macchia (Kemalpasa)	habitat pinetum (Belkave)
Quercus coccifera	+++	++
Phillyrea media	++	+
Jasminum fruticans	++	+
Cistus creticus	++	+++
Laurus nobilis	+	•
Paliurus spina-christi	+	•
Platanus orientalis	+	
Ruscus acutifolius	+	•
Pyrus amygdalifolius	+	
Cyclamen neapolitanum	++	•
Vitex agnus-castus	+	
Crataegus monogyna	++	+
Campanula lyrata	+	
Pistacia terebinthus	+	
Quercus ilex	+	•
Quercus infectoria	•	
Asparagus acutifolia	+	•
Cirsium spec.	•	
Rubus spec.	•	•
Taraxacum officinale	•	•
Origanum virens	•	
Marrubium spec.	•	
Rumex spec.	•	
Ranunculus arvensis	•	
Pinus halepensis	•	+++
Orchis analotica		+

Frequency very high +++
frequency high ++
frequency low +
rare •

Table 31: Results of hemagglutination inhibition tests with 173 human sera from West Cameroon.

Antigen	Number of positive sera		
	Group 1	Group 2	Total
Semliki Forest Disease	2	1	3
Sindbis	5	1	6
Yellow Fever	16	16	32
Murray Valley Encephalitis	31	17	48
West Nile	20	4	24
Dengue II	17	2	19
Tick-borne Encephalitis	5	0	5
T o t a l	40*/98	27*/75	67*/173

Number of sera positive/investigated

* Many of the sera showed cross reactions with more than one antigen

Table 32: Results of the neutralization tests (NT) with the 40 positive sera of group 1.

Virus	Positive reactions
O'nyongnyong	7
Chikungunya	12
Zika	2
Uganda S	14
T o t a l	22/40

Number of sera positive/investigated

Table 33: Titers of hemagglutination-inhibiting and neutralizing antibodies in the positive sera from residents of Cameroon.

Serum No.	Hemagglutination-inhibiting test							Neutralization test			
	SFD++	Sind.	YF	MVE	WN	D2	TBE	U'nyong	CH	Zika	U_3S
5						20		20			10
11			40*	640	160	80			40		10
20					10				40		10
21			80	640	80	160	40			10	
22			160	640	80	80					
25			10	40	20	20	10				20
32	40	40		160	80	20			40		
33				160	40	80			20		5
34		10	40	80	20	20		20			10
37				1280		20			10		20
42			1280	160	80	320				5	10
47				40				40			
48				10				10			5
50			40	40				10	10		
57			640	80	640	320	20	10			20
70		20							20		
71				20				10			
80				320	80	320					10
81			10	40	40	20			5		10
82	10	10	20			20			80		40
85							80		20		
90			80	80	40	20			40		10
92				40	80	320			10		

++ Abbreviations see Table 21 A

* Reciprocal titer of serum

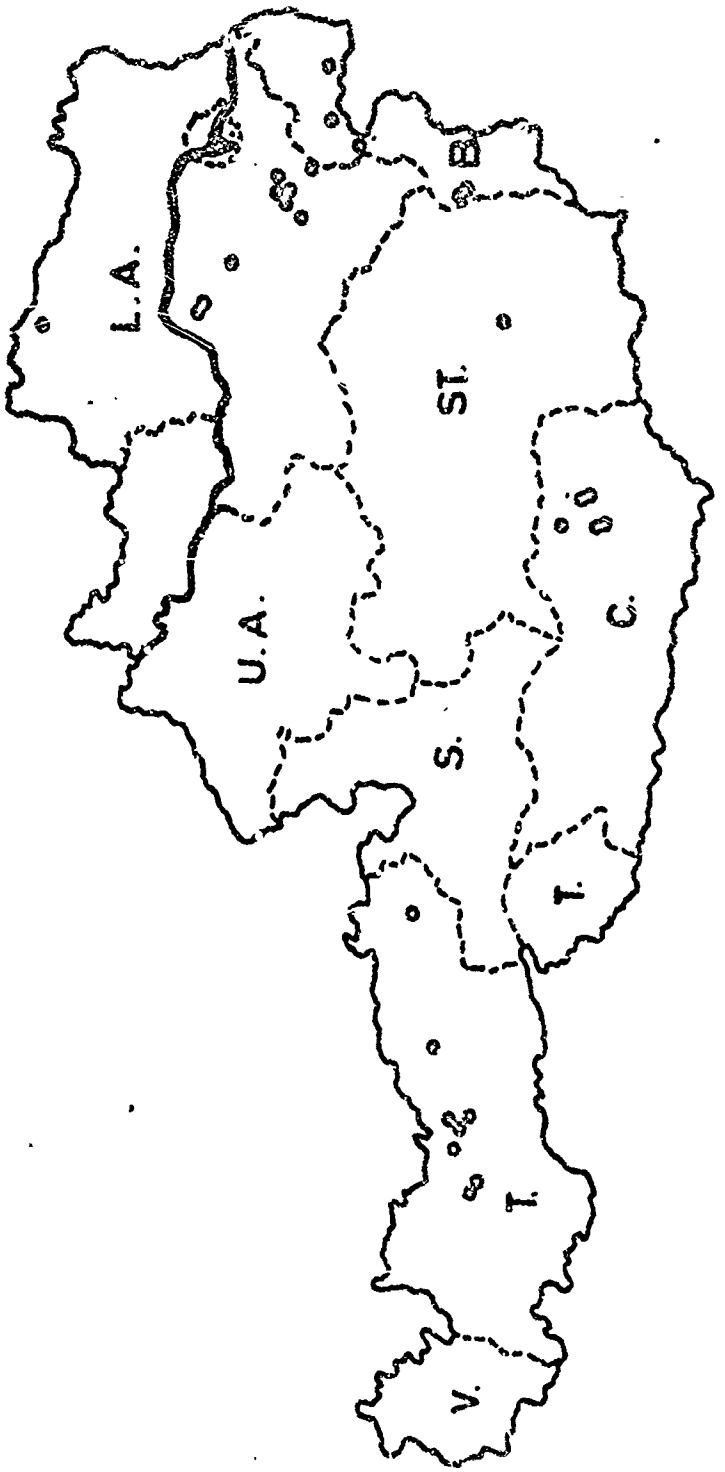


Fig.1: New foci of TBE detected in 1971 and 1972.

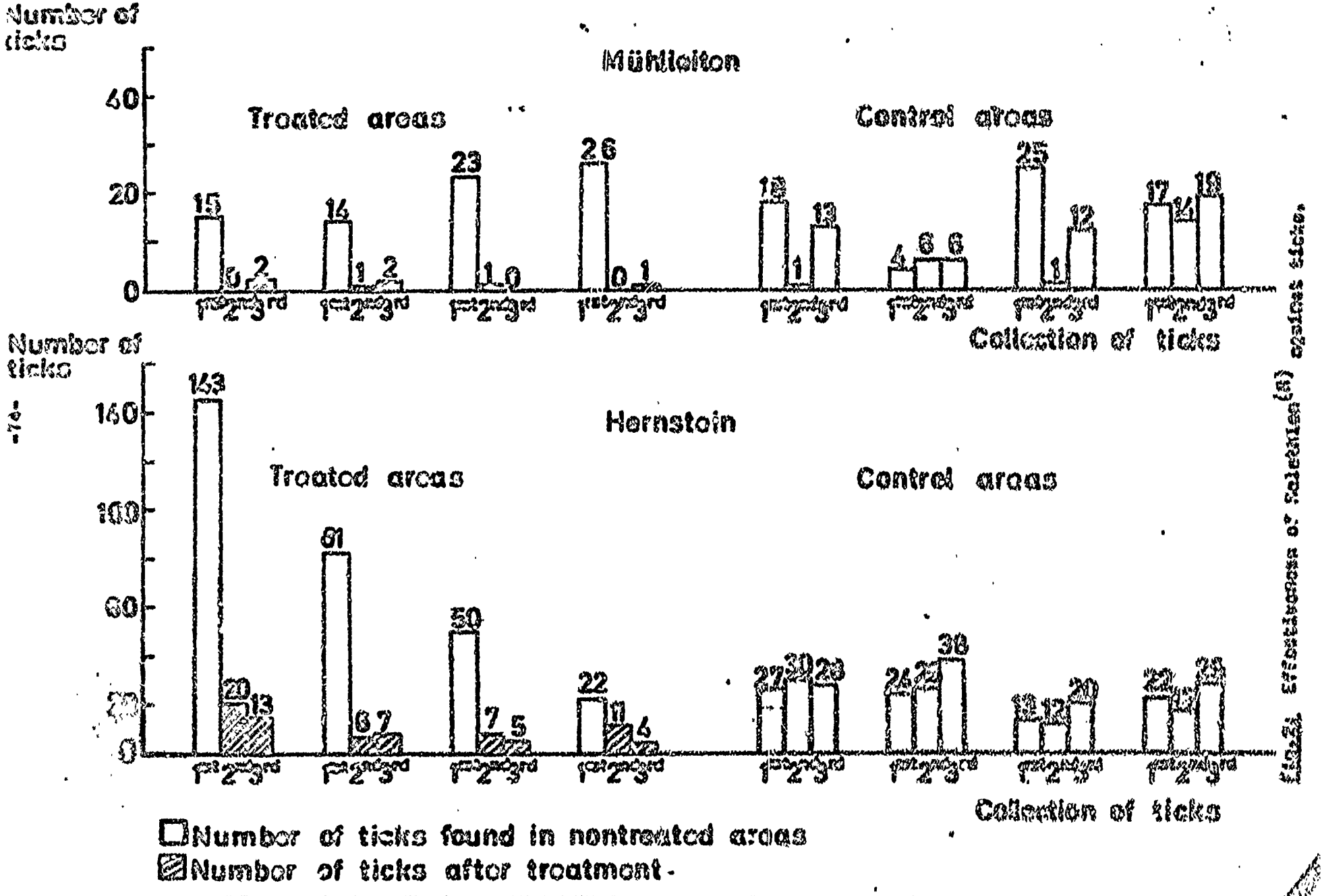


Fig. 5. Effectiveness of Folcemlen (S) against ticks.

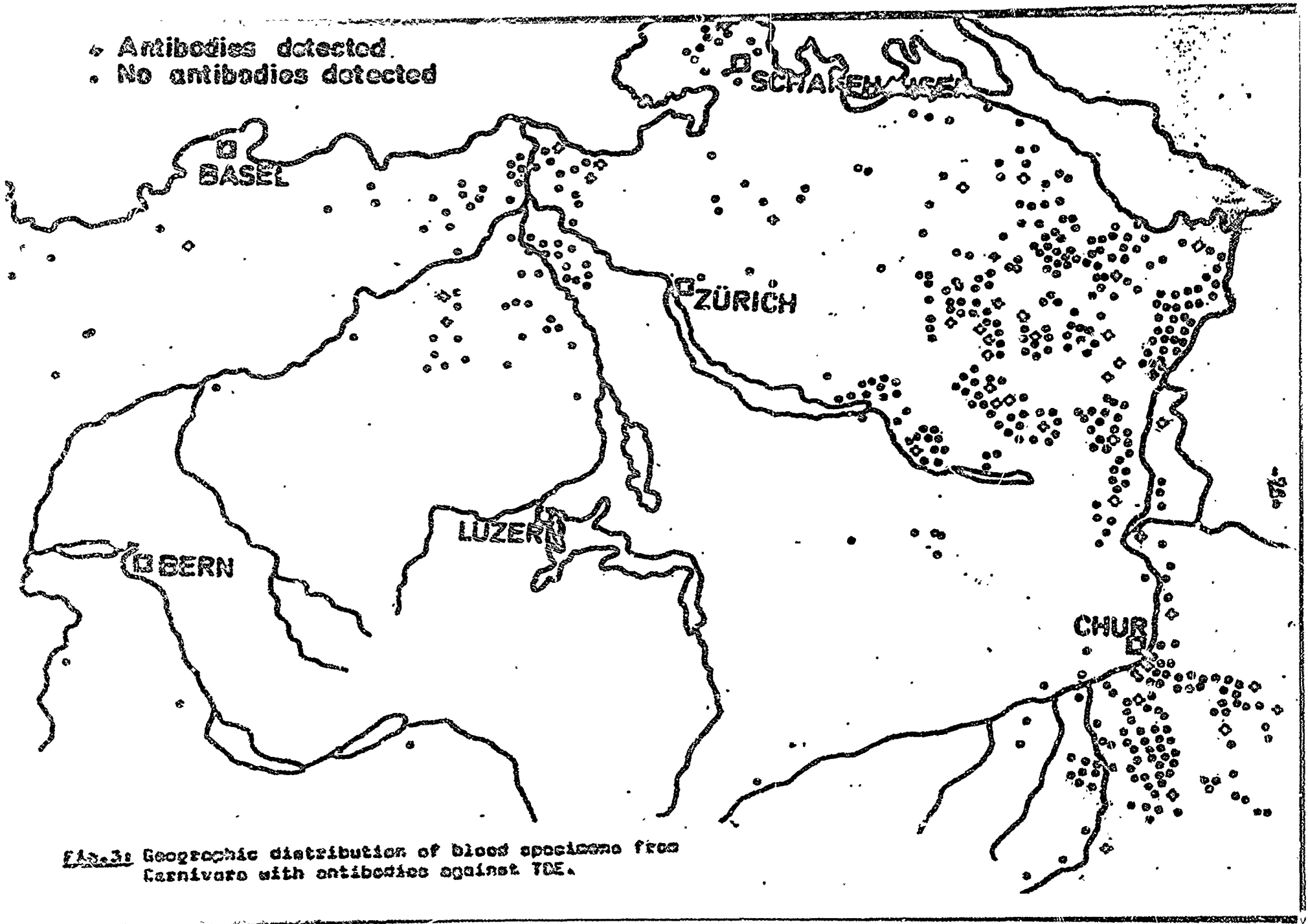


Fig. 3: Geographic distribution of blood specimens from Carnivora with antibodies against TBE.

Fig. 4: Separation of West Nile virus from proteinaceous impurities and from added albumin by CPG-chromatography.

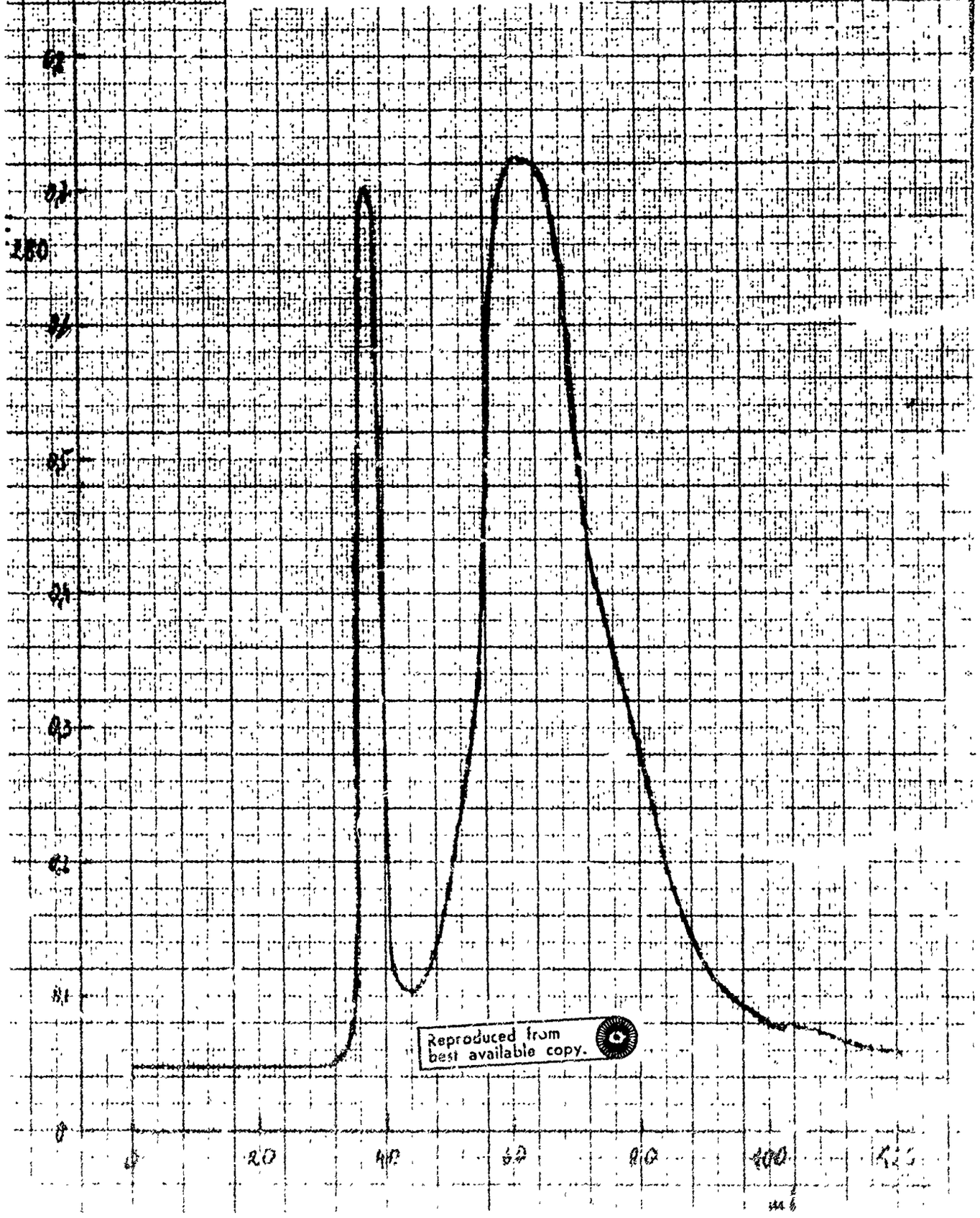


Fig. 51 Separation of the virus from protamin by CPG-chromatography

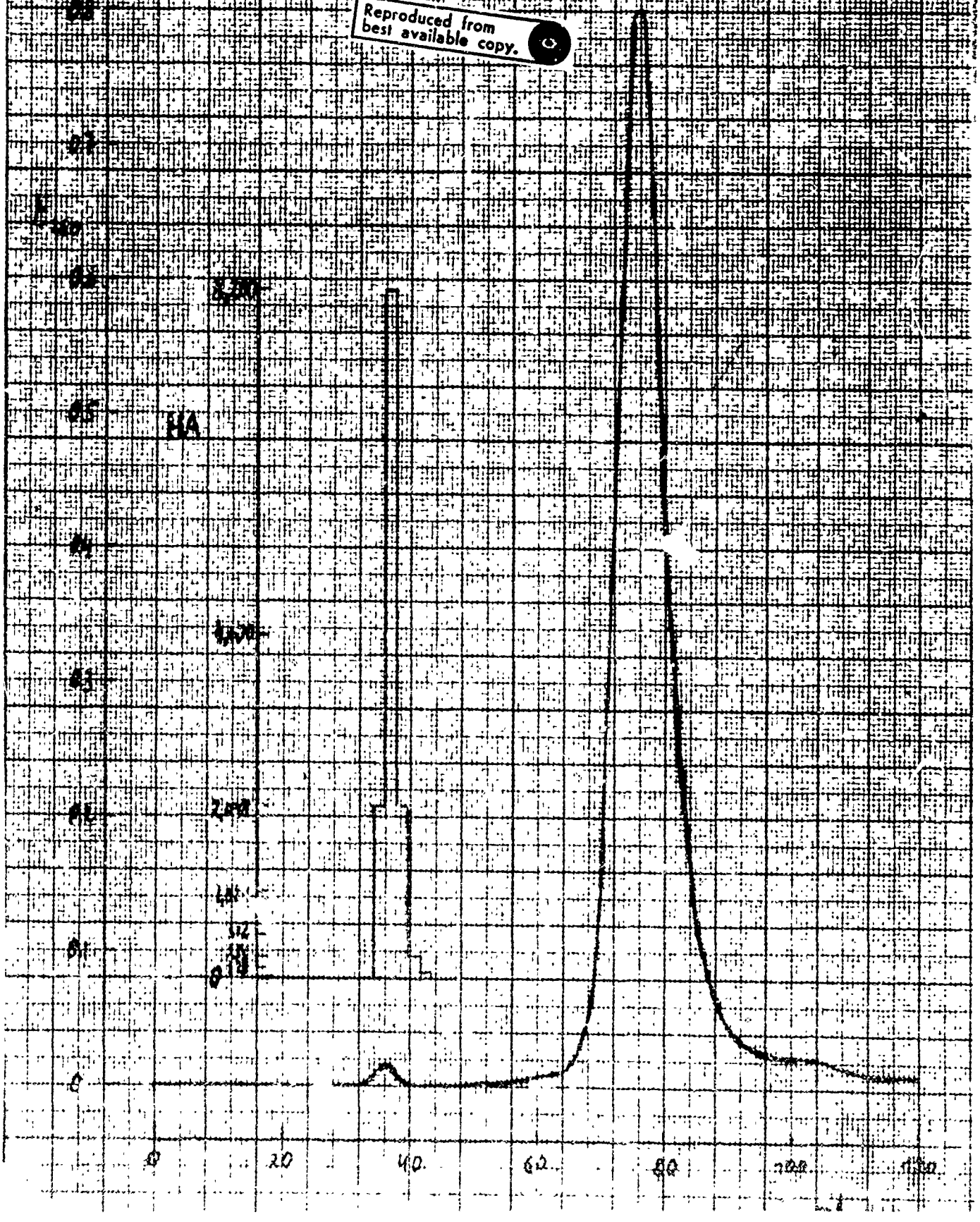
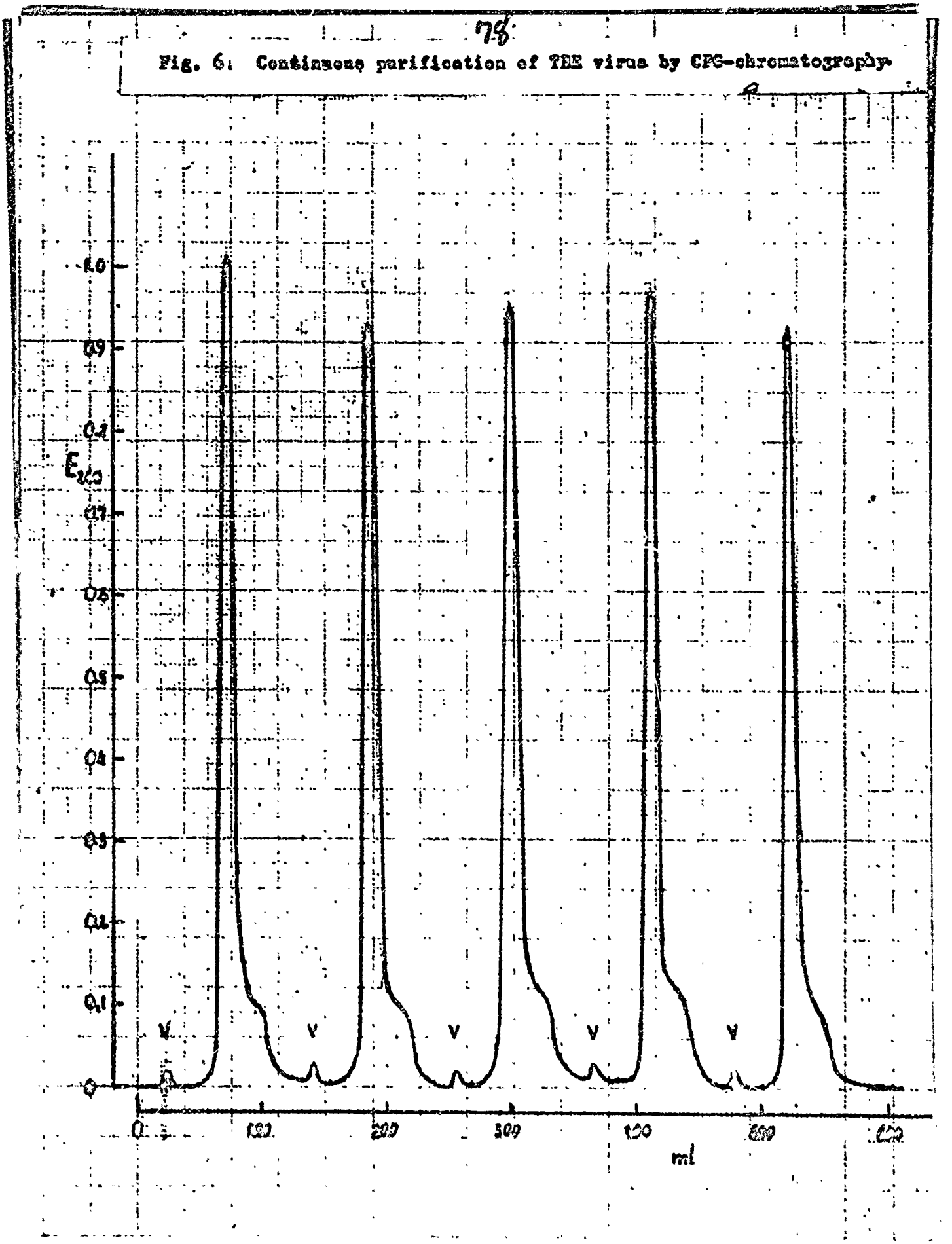


Fig. 6: Continuous purification of TBE virus by CPC-chromatography



KEY WORDS:

Arboviruses in Austria; eradication of TBE foci; TBE ecology; TBE in Switzerland; receptor for TBE (Synthesis); concentration of arboviruses; purification of arboviruses; persistent arbovirus infection; Langat virus; birds as hosts of arboviruses; arboviruses in Turkey; arboviruses in Cameroon.